REMARKS

The Office Action of July 30, 2008, contains a final rejection of the requirement for restriction. Applicants acknowledge this final rejection but respectfully request reconsideration. It is not ubiquinone, per se, which is the linking element, it is an oil/fat composition containing ubiquinone.

The Office Action further contains a plurality of prior art rejections. In that regard, Claims 15-18 were each rejected under 35 U.S.C. §102(e) over Udel ('942). Claim 16 was rejected under 35 U.S.C. §102(e) over Udel or, alternatively, under 35 U.S.C. §103(a) over Udel. Claim 18 was rejected under 35 U.S.C. §103(a) over Selzer in view of Udel.

Rejected independent Claim 15 has now been amended to more clearly define applicants process invention over the prior art relied upon. Previously presented Claims 16-18 depend from amended Claim 15. New Claims 23 and 24 also depend from amended Claim 15. For the reasons hereinafter set forth, applicants respectfully submit that amended Claims 15-18, 23 and 24 define process inventions which are neither anticipated, nor rendered obvious, by Udel or Selzer in view of Udel.

Amended Claim 15 is the only independent claim presently under consideration. Claim 15 has been amended to require a process in which ubiquinone is described in an oil/fat environment under heating at a temperature "of not lower than the melting points of ubiquinone". In contrast to the process of Claim 15, Udel teaches mixing of ubiquinone, MCT (medium-chain triglyceride) and other ingredients in an oil-wax mixture at a temperature within the range of 35°C to 45°C. The Udel process does not conduct mixing at a heating temperature of not lower than the melting points of ubiquinone, i.e., mixing at a heating temperature of not lower than about 48°C! Accordingly, amended Claim 15 is not anticipated by Udel.

Furthermore, Udel does not disclose a process of "dissolving ubiquinone" or any ubiquinone solution. The operative words used in Udel relevant to treatment of ubiquinone are "added", "mixed", "mixture", for example. Udel teaches, in column 3, lines 7 to 17 of the specification, that "Rice bran oil, a carrier suspension agent... such as Coenzyme Q_{10} is heated to 50 to 60°C." Thus, the object of Udel is to provide a ubiquinone-containing suspension. Actually, ubiquinone would not dissolve in the composition under conditions described by Udel. In other words, Udel does not "dissolve" ubiquinone in oil/fat by heating and, furthermore, the temperature condition described is quite different from that of the applicants' claimed invention. Once again, amended Claim 15 is not shown to be anticipated by Udel.

Referring further to Udel, the applicants' claimed process has the effect of preventing precipitation and/or localization of ubiquinone during storage of food by first adding ubiquinone in oil/fat to dissolve the ubiquinone under heating, and then, adding the resultant mixture to a food material. As a result, the food is not deleteriously affected by precipitation and/or localization of the critical ingredient, ubiquinone! These results, produced by applicants' Claim 15 process, are nowhere seen to be expected i.e., they are unexpected!

Still furthermore, regarding Udel, the Office Action stated that Claim 16 was anticipated, or rendered obvious, by Udel. Applicants respectfully submit that this conclusion is not supportable. As explained above, the claimed process invention produces the unexpected effect of preventing precipitation and/or localization of ubiquinone during storage of food by at first adding ubiquinone in oil/fat to dissolve under heating, in a specific condition, and then adding the resultant mixture to a food material.

In addition to the foregoing, the Examiner contends that a MCT has a melting point of not lower than 20°C. This means that a MCT is solid fat. But that cannot be true. Most MCTs have melting points of below 5°C, although the melting points depend on species. In particular, commercially-available MCTs have melting points within the range of -12°C to -5°C, and they are liquid. In that regard, the published article "Effect of Medium-chain Triacylglycerols on Anti-obesity Effect of Fucoxanthin" , which is attached, clearly discloses that MCTs are liquid at room temperature (see right column, page 1). The bottom line is that a person of ordinary skill in the relevant art would have a common sense understanding that MCT is liquid oil/fat, and that the use of oil/fat having a melting point of not lower than 20°C is neither inherently disclosed nor suggested to Udel by such a hypothetical person of ordinary skill.

Fatty acids that constitute commercial MCTs are a mixture of C6-C12 compounds, in which major components are C8 and C10 components. Such fatty acids are liquid oils. The "Myglyol" taught by Udel also contains C8 and C10 components as major components.

On the other hand, fatty acids that constitute coconut oil are not only lauric acid (C12) but also compounds having 14 or more carbon atoms, such as myristic acid or palmitic acid. Therefore, fatty acids that constitute coconut oil are solid fats that have melting points of 20°C or higher. Thus, coconut oil and a MCT are different from each other in their distribution and proportion of fatty acids that constitute coconut oil or a MCT. The fact that coconut oil has a melting point of 20°C or higher cannot be a basis of the presumption that a MCT has a melting point of 20°C or higher.

In addition, Udel '942 teaches, in column 3, lines 36 to 40, that bee's wax primarily increases viscosity to keep insoluble components from settling to one side of the soft gel capsule. Thus, the content of the capsule was logically fluid slurry, not solid.

The Examiner also states that Udel discloses a solidification process. However, the main ingredients of the Udel composition were rice bran oil and MCT, both of which are in a liquid form. Therefore, the recognition of a person skilled in the art is that the Udel's composition cannot be "solid", because Udel's composition was a suspension mixture of ubiquinone in oil/fat, which was finally encapsulated (filled) in a soft capsule. As explained above, the recognition of a person skilled in the art would be that MCT is liquid and, that a composition encapsulated in a soft capsule cannot be "solid". Thus, it cannot be said that Udel inherently teaches or suggests "solidification" using "oil/fat having a melting point of not lower than 20°C" in Claim 16.

Regarding the rejection of claims 15 - 18 under 35 U.S.C. §103 as being unpatentable over Selzer in view of Udel, Selzer does not teach or suggest a heating process. As explained above, the claimed invention enables ubiquinone to be added in oil drops without localization of ubiquinone, even if ubiquinone is enriched over solubility in oil/fat by preparing oil-in-water composition by dissolving ubiquinone in liquid oil/fat under heating at a temperature no lower than a melting point of ubiquinone and then cooling. In addition, the claimed invention thereby produces another effect, the effect that a ubiquinone-supplementation food does not cause solid-liquid separation, or localization, of ubiquinone after a lapse of time.

The aforedescribed effect of preventing localization of ubiquinone in the present invention is important for an ordinary food which is different from tablets or capsules. Thus, such an effect of the present invention could not be expected by Udel or Selzer, which relate to soft capsule preparations of liquid preparation. Selzer does not teach any solid fats. Selzer also does not teach any solid foods. As such, applicants submit that the present invention plainly could not have been obvious over Selzer in view of Udel to one of ordinary skill in the relevant art at the time of the invention.

In conclusion on subject of rejections on the merits, Claims 15-18, 23 and 24 each define inventions which are neither anticipated nor would they have been rendered obvious by Udel and/or Selzer. As such, these claims should each be in allowable form.

Regarding formal matters, it is noted that priority under 35 U.S.C. § 119 is not acknowledged in the Office Action. Applicants submit that the noted Japanese priority application was, in fact submitted in PCT Application No. PCT/JP03/00396, should be on file in the USPTO and should be acknowledged.

Respectfully submitted,

/Richard G. Lione/
Richard G. Lione
Reg. No. 19,795
Attorney for Applicant(s)

Attachment – Journal of Oleo Science publication

BRINKS HOFER GILSON & LIONE
P.O. Box 10395
Chicago, Illinois 60610
(312) 321-4200

Journal of Oleo Science

J. Oleo Sci. 56, (12) 615-621 (2007)

JOURNAL OF OLEO SCIENCE
JOS

Effect of Medium-chain Triacylglycerols on Anti-obesity Effect of Fucoxanthin

Hayato Maeda[1]*, Masashi Hosokawa[1], Tokutake Sashima[2], Katsura Funayama[3] and Kazuo Miyashita[1]

[1] *Faculty of Fisheries Sciences, Hokkaido University (Hakodate, Hokkaido 041-8611, JAPAN)*
[2] *Creative Research Institute, Hokkaido University (Hakodate, Hokkaido 041-8611, JAPAN)*
[3] *Riken Vitamin Co., (Tokyo 174-0065, JAPAN)*

Abstract: Dietary effects of medium-chain triacylglycerols (MCT) and fucoxanthin (Fc) on abdominal fat weight were determined using KK-Ay obese mouse. Experimental diet contained MCT(0.9%), Fc (0.1%), or MCT (0.9%)+Fc (0.1%). The abdominal fat weight of mice fed with Fc was significantly lower than that of mice fed with MCT. Uncoupling protein 1 (UCP1), a key molecule for metabolic thermogenesis, was clearly expressed in the white adipose tissue (WAT) of mice fed Fc, but little expression in that of the mice fed MCT. The anti-obesity effect of Fc was increased by mixing Fc with MCT. This increase would be due to the increase in the absorption rate of Fc by MCT.

Key words: fucoxanthin, obesity, medium-chain triacylglycerol

1 INTRODUCTION

Fucoxanthin is a characteristic carotenoid of brown seaweeds, such as *Undaria pinnatifida*, *Hijikia fusiformis* and *Sargassum fulvellum*. It has a unique structure including an allenic bond and 5,6-monoepoxide in the molecule. Anticarcinogenic effects, apoptosis induction on cancer cells, anti-inflammatory effects and radical scavenging activity are known as biological activities of fucoxanthin[1-4]. Furthermore, we have found that fucoxanthin shows anti-obesity effect with an interesting molecular mechanism[5]. Feeding with fucoxanthin significantly reduces white adipose tissue (WAT) in rats and mice with a clear expression of UCP1 protein and mRNA in WAT, while there was little expression of UCP1 in WAT in mice fed control diet. UCP1 is normally expressed only in brown adipose tissue (BAT). It dissipates the pH-grudient generated by oxidative phosphorylation, releasing chemical energy as heat. UCP1 expression in WAT by fucoxanthin intake leads to oxidation of fatty acids and heat production in WAT. The substrate oxidation would directly reduce WAT in animals. Considered as break- through discoveries for an ideal therapy for obesity, regulation of uncoupling protein 1 (UCP1) expression in adipose tissue by food constituent needs to be further explored.

Medium-chain triacylglycerols (MCT) are also being promoted as potential agents in the prevention of obesity[6]. MCT refers to mixed triacylglycerols of saturated fatty acids with chain length of 6-10 carbon[7]. Compared to long-chain triacylglycerols (LCT), MCT are smaller molecules and have a lower melting point, thus being liquid at room temperature[8]. The physico-chemical property of MCT tends to be more rapid and complete hydrolysis upon digestion as compared with that of LCT. The absorption and metabolism of MCT are also different from those of LCT. The products of MCT hydrolysis, monoacylglycerol and medium chain fatty acids (MCF), are rapidly absorbed through the stomach mucosa into the hepatic portal vein after ingestion. The medium chain fatty acids are bound to serum albumin and transported in the soluble form of fatty acids and enter to systemic circulation through the portal vein directly to the liver, without being incorporated in the chylomicron. They do not accumulate in adipose tissue or muscle. Since MCF leave the intestinal mucosa by the portal vein system, they reach the liver more rapidly compared to the longer molecules[8]. In addition, transportation of the MCF into the mitochondria and its β-oxidation rate is more rapid than those of long chain fatty acids. These characteristic digestion, absorption, and metabolism of MCT are recognized to be due to the increased energy expenditure and thus reducing weight gain in the animals

*Correspondence to: Hayato Maeda, Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041-8611, JAPAN
E-mail: hayatosp@fish.hokudai.ac.jp (H. Maeda)
Accepted August 21, 2007 (received for review May 2, 2007)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://jos.jstage.jst.go.jp/en/

fed MCT[8]. Human studies have also shown an increase in energy expenditure with regard to consuming MCT containing meals[9,10].

In the present study, we investigated anti-obesity effects of mixtures of fucoxanthin and MCT using KK-Ay obese mouse. Fucoxanthin is soluble in MCT or in fish oil at room temperature, while solubility of fucoxanthin in vegetable oil is very low. Antioxidants such as vitamin E (Vit.E) are generally used in the products of unstable carotenoids such as astaxanthin and fucoxanthin to protect against oxidation. However, solubility of fucoxanthin into Vit.E is very low. In the present study, we could easily mix fucoxanthin with Vit.E using MCT as a medium.

2 MATERIALS AND METHOD

2.1 Fucoxanthin and MCT

Dried powder of wakame (*Undaria pinnatifida*) was obtained from Riken Vitamin (Tokyo, Japan). Wakame lipid obtained by extraction of powder with acetone was subjected to silicic acid column chromatography using n-hexane/acetone (7 : 3, v/v) as mobile phase to separate fucoxanthin (Fc). The separation was repeated three more times for further purification of Fc. Isolated Fc showed a purity of more than 78% on high performance liquid chromatography (HPLC). HPLC was carried out using Hitachi L-7000 system with the reversed - phase column (Develosil ODS-UG-5; 250 × 4.6 mm i.d., 5.0-μm particle size, Nomura Chem. Co.) fitted with a 10 × 4.0 mm i.d. guard column containing the same stationary phase. A mixture of methanol and acetonitrile (70 : 30, v/v) at a flow rate of 1.0 mL/min was used as mobile phase. Fc was monitored at 450 nm using UV-Vis detector. Peak identification was carried out by the comparing with standard. The standard fucoxanthin was prepared as previously described (1). MCT and Vit.E mixture were kindly donated by Riken Vitamin. Main fatty acids of MCT were caprylic acid (C8 : 0; 58.3%) and capric acid (C10 : 0; 41.5%).

2.2 Animals and diets

Female KK-Ay mouse (3weeks old) was obtained from Japan CREA Co. (Tokyo, Japan). They were housed at controlled temperature (23 ± 1℃) and humidity (50%) room under a 12 : 12-h light-dark cycle. After acclimation for 1 week by feeding control diets, mice were randomly divided into three groups of seven mice and given free access to water and the experimental diet. The diet was prepared according to the recommendation of the American Institute of Nutrition (AIN-93G)[12]. Composition of experiment diets are shown in Table 1. Fatty acid composition of dietary fat are shown in Table 2. After feeding of experimental diets for 4 weeks, mice were starved for 12 hours and anesthetized with diethyl ether. Mice were killed by exsanguination and their blood was withdrawn at the abdominal artery. Abdominal WAT, BAT, and liver were rapidly removed and weighed. Both adipose tissues and liver were frozen in liquid nitrogen for Western blot analy-

Table 1 Composition of Experimental Diets (in grams).

	Group		
Ingredients	MCT	Fc	Fc + MCT
Soybean oil[1]	125.10	134.10	124.10
Medium-chain triglyceride[2]	9.00		9.00
Vitamin E[2]	1.00		1.00
Fucoxanthin (Fc)		1.00	1.00
Corn starch[3]	346.28	346.28	346.28
Casein[3]	216.00	216.00	216.00
Dextrinized cornstarch[3]	114.99	114.99	144.99
Sucrose[4]	87.12	87.12	87.12
AIN-93 mineral mixture[3]	35.00	35.00	35.00
AIN-93 vitamin mixture[3]	10.00	10.00	10.00
L-cystine[3]	3.00	3.00	3.00
Choline bitartrate[3]	2.50	2.50	2.50
Cellulose[3]	50.00	50.00	50.00
Tert-Butyl hydroquinone[5]	0.01	0.01	0.01

[1] Wako Pure Chemical Ind., Osaka, Japan
[2] Riken vitamin corporation, Tokyo, Japan
[3] CLEA Japan, Tokyo, Japan
[4] Kanto kagaku Reagent division, Tokyo, Japan
[5] SIGMA-ALDRICH Japan, Tokyo, Japan

Table 2 Fatty Acid Composition of Dietary Fat.

Fatty acid (wt %)	Dietary fat		
	MCT	Fc	Fc+MCT
8:0	1.7	N.D[a]	1.9
10:0	1.5	N.D[a]	1.8
16:0	10.0	10.7	10.1
18:0	3.6	3.7	3.6
18:1n-9	23.3	23.7	23.2
18:1n-7	1.4	1.6	1.7
18:2n-6	50.0	51.2	49.8
18:3n-3	5.5	5.8	5.4
18:4n-3	N.D[a]	N.D[a]	N.D[a]
20:5n-3	N.D[a]	N.D[a]	N.D[a]

N.D[a]: Not detected

sis and enzymatic activity measurement, respectively. The study protocol was approved by the committee of Hokkaido University.

2.3 Fatty acid composition of dietary fat

The fatty acid composition of the dietary fat was determined by gas chromatography (GC). After conversion of fatty acyl groups in the fat to their methyl esters as described previously by Prevot and Mordet[12], GC analysis was performed on a Shimadzu GC-14B (Shimadzu Seisakusho Co, Ltd, Kyoto, Japan) equipped with a flame-ionization detector and a capillary column [Omegawax 320 (30 m × 0.32 mm i.d.); Supelco, Inc., Bellefonte, PA]. The injection port and flame ionization detector were operated at 250 and 260℃, respectively. The column temperature was held at 200℃. Component peaks were identified by comparison with standard fatty acid methyl esters and quantified by a Shimadzu Chromatopac C-R6A integrator (Shimadzu Seisakusho Co., Ltd.).

2.4 Analysis of blood and liver

Adiponectin and Leptin level of the blood plasma were analyzed by commercial ELISA kit, namely, Mouse Adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd. Tokyo Japan) and Mouse Leptin Assay Kit (L)-IBL (IBL Co., Ltd. Gunma Japan), respectively.

Liver (50 mg) was homogenized by a Polytron in 1 mL Tris buffer saline (20 mM Trizma base, 137 mM NaCl, pH 7.6). Triacylglycerol concentration and protein concentration were analyzed by enzymatic kits. Triglyceride E-test Wako (Wako Pure Chemical Industries, Osaka, Japan) and DC protein assay kit (Bio-Rad Laboratories, California USA) were used for this purpose. For the analysis of lipid metabolic enzyme activity, liver (*ca.* 300 mg) was homogenized with 7 volume of 0.25 mol/L sucrose and centrifuged at 500 × g for 10 min[16]. The supernatant was used for analysis of Carnitine palmitoyl transferase activity according to the method described by Ide *et al.*[13]. The supernatant was further centrifuged at 9000 × g for 10 min to isolate mitochondria. Glucose-6-phosphate dehydrogenase activity of this fraction was measured as described by Keley and Kletzien[16].

2.5 Western blot analysis

Each tissue was homogenized in 5-10 volume of a solution containing 10 mM Tris-HCl, and 1 mM EDTA (pH 7.4) for 30s with a Polytron. After centrifugation at 1500 g for 5 min, the fat cake was discarded, and the infranatant (fat-free extract) was used for Western blot analysis of UCP1 as described previously[17]. Total protein content in BAT and WAT was measured with a DC protein assay kit (Bio-Rad Laboratories, California USA). Supernatants (30 μg protein/lane) were separated by 10% SDS-polyacrylamide gel electrophoresis, and proteins were transferred to polyvinylidene difluoride membrane. The membrane was incubated with UCP 1 antibody (Sigma, Saint Louis, USA) for one hour and then was incubated with a secondary antibody rabbit IgG-conjugated horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for one hour at room temperature. The membrane was treated with the reagents in the chemiluminescence detection kit (ECL system, Amersham Pharmacia Biotech, Piscataway, NJ, USA) according to the manufacturer's instructions. β-Actin was detected as a control with β-Actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

2.6 Statistical analysis

The level of significance for differences between the groups was tested using one-way ANOVA and the Student's *t*-test.

3 RESULTS

3.1 Body weight and adipose tissue weight

All animals remained healthy though the experimental period. There was no significant difference ($P > 0.05$) in body weight and in energy intake between the groups (Fig. 1). Uterinary and mesentery WAT significantly ($P < 0.05$) decreased by the supplementation of fucoxanthin (Fc) or Fc +MCT as compared with MCT group (Fig. 2). This effect was found more clearly in Fc+MCT than that in Fc alone. On the other hand, BAT content was significantly ($P < 0.01$) higher in the mice fed Fc or Fc+MCT than in the mice fed MCT diet (Fig. 2).

3.2 Adiponectin and leptin level of blood plasma, liver lipid content, and enzymatic activity

There was no significant difference ($P > 0.05$) in the adiponectin level of blood plasma (Fig. 3). Diet with Fc +

MCT significantly ($P<0.05$) reduced the leptin level of blood plasma as compared with MCT diet, while no significant difference ($P>0.05$) in the leptin level was found between Fc group and MCT group. Liver triacylglycerol concentration decreased in Fc group and Fc+MCT group as compared with that in MCT group, but significant difference ($P<0.05$) was only found in Fc (Table 3). Carnitine palmitoyltransferase activity increased by feeding Fc or Fc +MCT, but not significantly ($P>0.05$). Glucose-6-phosphate dehydrogenase activity did not change in each group.

3.3 UCP1 protein expression in BAT and WAT

There was no difference in the UCP1 protein expression in BAT among the three groups (Fig. 4). On the other hand, UCP1 expression in WAT increased by feeding Fc and significant increase ($P<0.05$) was found in Fc+MCT group (Fig. 5).

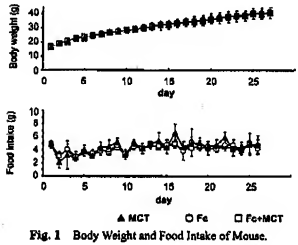

Fig. 1 Body Weight and Food Intake of Mouse.

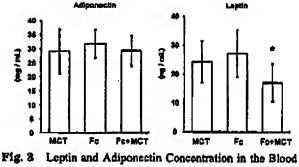

Fig. 3 Leptin and Adiponectin Concentration in the Blood Plasma. *$P<0.05$ vs MCT

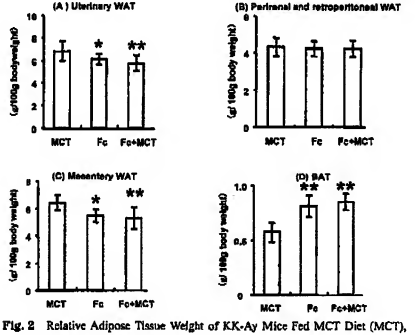

Fig. 2 Relative Adipose Tissue Weight of KK-Ay Mice Fed MCT Diet (MCT), 0.1% Fc diet (Fc), 0.1% Fc +MCT diet.
(A) Uterine WAT , (B) Perirenal and retroperitoneal WAT, (C) Mesentery WAT, (D) Brown adipose tissue *$P<0.05$ vs MCT **$P<0.01$ vs MCT

Table 3 Lipid Content and Enzyme Activities in the Liver.

	Group		
	MCT	Fc	Fc+MCT
Liver triacylglycerol (mg/g protein)	238±64	167±33*	205±46
Carnitine palmitoyl transferase (μmol/min/mg protein)	2.1±0.7	2.6±0.6	2.5±0.6
Glucose-6-phosphate dehydrogenase (μmol/min/mg protein)	2.6±0.5	2.6±0.3	2.9±0.8

*$P<0.05$ vs MCT

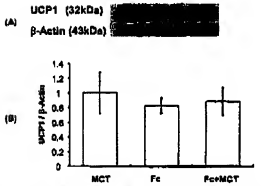

Fig. 4 (A) Western Blot Analysis of Uncoupling Protein1 (UCP1) in BAT. (B) Level of UCP1 Protein Expression Relative to β-Actin.

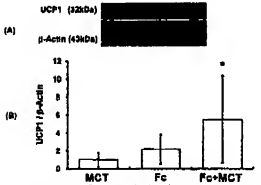

Fig. 5 (A) Western Blot Analysis of Uncoupling Protein1 (UCP1) in WAT. (B) Relative Expression Level of UCP1 Protein to β-Actin. *$P<0.05$ vs MCT

4 DISCUSSION

The increasing incidence of obesity is becoming a medical problem in the world. Weight loss can be achieved by different means but the maintenance after weight loss in the long term is rarely shown. Therefore, identification of substances that can decrease or prevent obesity remains a requirement. MCT has different physical properties as compared with long-chain triacylglycerols, with MCT showing a greater increase in energy expenditure in animal studies and human studies[18]. Thus, MCT can be useful agents in the prevention of obesity. On the other hand, a great deal of interest has been focused on adaptive thermogenesis by UCP1 as a physiological defense against obesity[19,20] UCP1 is a key molecule for anti-obesity. UCP1 expression is known to be a significant component of whole body energy expenditure, and its dysfunction contributes to the development of obesity. In a previous study on the anti-obesity effect of fucoxanthin, we have found that fucoxanthin reduced abdominal fat accumulation through UCP1 expression in WAT[9]. In the present study, we used three dietary fat containing MCT (0.9%), Fc (0.1%), MCT (0.9%)+Fc (0.1%). As shown in Fig. 2, the weights of uterinary and mesentery WAT were significantly lower in Fc-fed mice than in MCT-fed mice. This result indicates the stronger anti-obesity effect of Fc (0.1%) than MCT (0.9%). We have found the clear expression of UCP1 protein and mRNA in WAT of KK-Ay mice fed Fc (0.4%)[9]. The same result was also observed in WAT of mice fed Fc (0.1%) (Fig. 5), while little expression in that of MCT-fed mice. Furthermore, BAT weight was significantly higher in Fc-fed mice than in MCT-fed mice (Fig. 2). UCP1 is generally known to be expressed in BAT. Although there was no significant difference in BAT content, increase in BAT of mice fed Fc would affect the reduction of abdominal fat of the mice. Therefore, the energy dissipation via the generation of heat by UCP1 expression would be more effective on the reduction of abdominal fat than the enhancement of energy expenditure induced by MCT feeding.

In most studies on the anti-obesity effect of MCT, the concentration of MCT in the diet is around 5%. The lower effect of MCT in the present study would be due to the lower concentration of MCT (0.9%). On the other hand, the effect of Fc on the reduction of abdominal fat increased by mixing Fc with the low concentration of MCT (Fig. 2).

UCP1 expression of mice fed Fc+MCT was higher than those of mice fed Fc or MCT alone (Fig. 5). Leptin is secreted from adipocyte and adjusts the body weight or insulin sensitivity[23]. Leptin suppresses the appetite to control body weight. However, obesity patient has leptin resistance and leptin level in the blood keeps high in the patient. Therefore, plasma leptin level is used as an index of body fat accumulation. Leptin level of obese model mice (KK-Ay) fed Fc + MCT was significantly lower than those of other mice fed MCT and Fc alone.

Dietary fucoxanthin is converted to fucoxanthinol after absorption[22]. Absorption rate is generally affected by the composition of food matrix. We found that the solubility of Fc in soybean oil is very low, while Fc can easily dissolve in MCT or in fish oil. MCT diet also contained Vit.E (0.1%). Vit.E might be effective for the prevention of Fc against oxidation and/or decomposition. Thus, the higher anti-obesity effect of Fc with MCT than Fc alone would be due to the increase in the absorption rate of Fc and in the oxidative stability of Fc.

When the UCP1 expression in WAT was compared between mice fed with purified fucoxanthin and seaweed lipids containing fucoxanthin, the higher UCP1 level was found in the mice fed seaweed lipids than those fed purified fucoxanthin, although the fucoxanthin content was the same in both groups (unpublished data). This suggests that the absorption rate of fucoxanthin is strongly affected by the presence of other components, especially lipids. The synergistic relationship between Fc and other lipids need to be clarified by further study.

ACKNOWLEDGMENT

This work was partially supported by Research and Development Program for New Bio-industry Initiatives and the Grant-in-Aid for Scientific Research and the 21st century COE Program "Marine Bio-Manipulation Frontier for Food Production" of Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1. Hosokawa, M., Wanezaki, S., Miyauchi, K., Kurihara, H., Kohno, H., Kawabata, J., Odashima, S., Takahashi, K. Apoptosis-inducing effect of fucoxanthin on human leukemia cell line HL-60. *Food Sci. Technol. Res.* 5, 243-246 (1999).
2. Ikeda, K., Kitamura, A., Machida, H., Watanabe, M., Negishi, H., Hiraoka, J., Nakano, T. Effect of *Undaria pinnatifida* (Wakame) on the development of cerebrovascular diseases in stroke-prone spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol.* 30, 44-48 (2003).
3. Murakami, A., Nakashima, M., Koshiba, T., Maoka, T., Nishino, H., Yano, M., Sumida, T., Kim O.K., Koshimizu K., Ohigashi H. Modifying effects of carotenoids on superoxide and nitric oxide generation from stimulated leukocytes. *Cancer Lett.* 28, 115-123 (2000).
4. Okuzumi, J., Nishino, H., Murakoshi, M., Iwashima, A., Tanaka, Y., Yamane, T., Fujita, Y., Takahashi, T. Inhibitory effects of fucoxanthin, a natural carotenoid, on N-myc expression and cell cycle progression in human malignant tumor cells. *Cancer Lett.* 19, 75-81 (1990).
5. Maeda, H., Hosokawa, M., Sashima, T., Funayama, K., Miyashita, K. Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem. Biophys. Res. Commun.* 332, 392-397 (2005).
6. St-Onge, M.P., Jones, P.J. Physiological effects of medium-chain triglycerides: potential agents in the prevention of obesity. *J. Nutr.* 132, 329-332 (2002).
7. Marten, B., Pfeuffer, M., Schrezenmeir, J. Medium-chain triglycerides. *Int. Dairy J.* 16, 1374-1382 (2006).
8. Papamandjaris, A.A., MacDougall, D.E., Jones, P.J. Medium chain fatty acid metabolism and energy expenditure: obesity treatment implications. *Life Sci.* 62, 1203-1215 (1998).
9. Lasekan, J.B., Rivera, J., Hirvonen, M.D., Keesey, R.E., Ney, D.M. Energy expenditure in rats maintained with intravenous or intragastric infusion of total parenteral nutrition solutions containing medium- or long-chain triglyceride emulsions. *J. Nutr.* 122, 1483-1492 (1992).
10. Hill, J.O., Peters, J.C., Yang, D., Sharp, T., Kaler, M., Abumrad, NN., Greene, HL. Thermogenesis in humans during overfeeding with medium-chain triglycerides. *Metabolism* 38, 641-648 (1989).
11. White, M.D., Papamandjaris, A.A., Jones, P.J. Enhanced postprandial energy expenditure with medium-chain fatty acid feeding is attenuated after 14 d in premenopausal women. *Am. J. Clin. Nutr.* 69, 883-889 (1999).
12. Reeves, P.G., Nielsen, F.H., Fahey, G.C. Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* 123, 1939-1951 (1993).
13. Pravot, A.F., Mordret, F.X. Utilisation des colonnes capillaries de verre pour l'analyse des corps gras par chromatographie en phase gazeuse. *Rev. Fse. Corps Gras.* 23, 409-423 (1976).
14. Murata, M., Sano, Y., Ishihara, K., Uchida, M. Dietary fish oil and *Undaria pinnatifida* (wakame) synergistically decrease rat serum and liver triacylglycerol. *J. Nutr.* 132, 742-747 (2002).
15. Ide, T., Watanabe, M., Sugano, M., Yamamoto, I. Activi-

ties of liver mitochondrial and peroxisomal fatty acid oxidation enzymes in rats fed trans fat. *Lipids* 22, 6-10 (1987).
16. Kelley, D.S.; Kletzien, R.F. Ethanol modulation of the hormonal and nutritional regulation of glucose 6-phosphate dehydrogenase activity in primary cultures of rat hepatocytes. *Biochem. J.* 15, 543-549 (1984).
17. Nagase, I.; Yoshida, T.; Kumamoto, K.; Umekawa, T.; Sakane, N.; Nikami, H.; Kawada, T.; Saito, M. Expression of uncoupling protein in skeletal muscle and white fat of obese mice treated with thermogenic β3-adrenergic agonist. *J. Clin. Invest.* 97, 2898-2904 (1996).
18. Che, Man, Y.B.; Manaf, M.A. Medium-chain triacylglycerols in *Nutraceutical and Specialty Lipids and Their Co-products*. (Shahidi, F. ed.). Taylor & Fransis, Boca Roton, FL, pp. 27-56 (2006).
19. Dulloo, A.G.; Samec, S. Uncoupling proteins: their roles in adaptive thermogenesis and substrate metabolism reconsidered. *Br. J. Nutr.* 86, 123-139 (2001).
20. Jezek, P. Possible physiological roles of mitochondrial uncoupling proteins--UCPn. *Int. J. Biochem. Cell Biol.* 34, 1190-1206 (2002).
21. Matsuzawa, Y. The metabolic syndrome and adipocytokines. *FEBS Lett.* 22, 2917-2921 (2006).
22. Sugawara, T.; Baskaran, V.; Tsuzuki, W.; Nagao, A. Brown algae fucoxanthin is hydrolyzed to fucoxanthinol during absorption by Caco-2 human intestinal cells and mice. *J. Nutr.* 132, 946-951 (2002).